

Description of the female, egg and first instar nymph of the stick insect *Paraphasma paulense* (Phasmatodea: Pseudophasmatidae) from Southeast Brazil

PEDRO IVO CHIQUETTO-MACHADO¹, FABIANO FABIAN ALBERTONI¹

¹ Museu de Zoologia, Universidade de São Paulo. Avenida Nazaré, 481. São Paulo, SP, Brazil. CEP 04263-000.

Corresponding author: *Pedro Ivo Chiquetto-Machado* (pedroivocm@gmail.com)

Academic editor: *Matan Shelomi* | Received 31 May 2017 | Accepted 11 August 2017 | Published 13 October 2017

<http://zoobank.org/712F2276-0C6B-4582-B753-E9583A7EA160>

Citation: Chiquetto-Machado PI, Albertoni FF (2017) Description of the female, egg and first instar nymph of the stick insect *Paraphasma paulense* (Phasmatodea: Pseudophasmatidae) from Southeast Brazil. Journal of Orthoptera Research 26(2): 91–101. <https://doi.org/10.3897/jor.26.20180>

Abstract

The stick insect *Paraphasma paulense* was described based on one male from the vicinity of São Paulo city, state of São Paulo, Brazil. Here we describe and illustrate the female, egg and first instar nymph of the species, including scanning electron micrographs of the egg. In addition, we give brief behavioural reports and present a few comments on the biology and morphology of *P. paulense*.

Key words

Atlantic Forest, biology, morphology, neotropical, Stratocleinae, Stratocleini

Introduction

Phasmatodea is one of the most poorly studied insect orders in Brazil. Most of the information on stick insects from the country is limited to original descriptions of species, mainly in the works by Gray (1835), Burmeister (1838), Westwood (1859), Bates (1865) and Kirby (1904), in the monograph by Brunner von Wattenwyl (1907) and Redtenbacher (1906, 1908), and in various papers by Toledo Piza (1936, 1937, 1938, 1944, among others). Recently, only a few studies have addressed Brazilian stick insects, such as: three broad taxonomic revisions that included some Brazilian taxa (Zompro 2001, 2004, Hennemann et al. 2016); the redescription of *Tithonophasma tithonus* (Gray, 1835) (Lima et al. 2013); and the descriptions of one new species in the genus *Cladomorphus* Gray, 1835 (Kumagai and Fonseca 2009), and another in *Agrostia* Redtenbacher, 1906 (Heleodoro et al. 2017). Furthermore, few Brazilian species have the egg described in spite of the importance of egg morphology and egg-laying mechanism in Phasmatodea taxonomy and phylogenetics (Sellick 1997, Clark Sellick 1998, Zompro 2004, Goldberg et al. 2015).

Paraphasma Redtenbacher, 1906 is one of the 15 genera belonging to the monotribal subfamily Stratocleinae (Pseudophasmatidae). This subfamily is restricted to the Neotropics and is composed of small- to medium-sized stick insects, usually with body and legs of delicate aspect, antennae frequently surpassing body length, short tegmina, and both sexes with well-developed hind wings.

Paraphasma currently contains ten species of which eight are recorded from Brazil (Zompro 2004, Brock et al. 2017). The de-

scription of the genus was based on characters of the femora, tibiae, tegmina and mesosternum (Redtenbacher 1906). No clear synapomorphy is known for *Paraphasma*, and it is defined by a vague set of diagnostic characters: profemur with anterodorsal carina distinctly raised (especially in female) and ventromedian carina acute; femora and tibiae of second and third legs with indistinct edges; tegmina short and scale-shaped, bearing distinct and often pointed shoulders; and variegated coloration in shades of yellow or brown (Redtenbacher 1906, Zompro 2004).

Paraphasma paulense Rehn, 1918 was described based on a single male collected in Cantareira, near the city of São Paulo, São Paulo state, Southeast Brazil. The author provided a considerably detailed description for the species and regarded it as related to *Paraphasma quadratum* (Bates, 1865) based on the relatively long tegmina of both species. However, taxonomic status of the species currently assigned to *Paraphasma* requires revision, and *P. paulense* will be soon removed from this genus as a result of an ongoing taxonomic study (Oskar Conle, pers. comm.).

Here we present a contribution to the knowledge of Brazilian stick insects, describing and illustrating the egg, first instar nymph and adult female of *P. paulense*, and also providing some comments on biological and morphological aspects of the species.

Material and methods

Collecting locality.— The collections were conducted in the Boraceia Biological Station (BBS) (23°39'14"S, 45°53'21"W), municipality of Salesópolis, São Paulo state, Southeast Brazil. The BBS is a research station maintained by the Museum of Zoology of University of São Paulo (MZSP) within the Serra do Mar State Park, which is the largest Atlantic Rain Forest fragment in Brazil, with 332,000 ha (Fundação Florestal 2017). The vegetation around the collecting area is predominantly composed of palm trees (especially *Euterpe edulis* Mart.), ferns and bamboos; in the soil abound heliconias and bromeliads.

Procedures.— Specimens were collected using a light trap by the white wall of the BBS building. Adults were maintained in a plastic container of 34×24 cm and 22 cm high, with water-soaked cotton and branches with leaves of at least three plant species at a time. Eggs were fixed in boiling water; nymphs were fixed as soon

as they died. Both were preserved in 80% ethanol; adults were pinned and dried.

Identification of specimens was made by Fernando De Domenico (PhD at MZSP) and the first author, through the key available in Zompro (2004), and by comparison of the collected males with the original description (Rehn 1918) and with the image of the holotype in Brock et al. (2017).

Specimens and eggs were examined under a stereomicroscope Leica M125 and measured with a digital calliper or under stereomicroscope with ocular micrometer. Three eggs had their opercula detached and shells destroyed to allow examination and measurement of the operculum and internal micropylar plate. When different measures were obtained, we gave the highest and lowest value (Tables 1–2). Photographs were taken with a camera Canon 5D mark II coupled to a Canon macro lens MP-E 65mm 1–5 $\times$, with a camera Zeiss MRc5 coupled to a stereomicroscope Zeiss SteREO Discovery.V12, and with an iPhone 4S.

Scanning Electron Micrographs (SEM) of the egg were made at the MZSP laboratory with a Carl-Zeiss microscope LEO 440. The egg was cleaned manually with a brush in a water and detergent solution. For embryo scanning, the egg was submitted to a critical point dryer, the shell was ripped out and the embryo was gold covered for a first scanning; secondly the membranes enclosing the embryo were ripped out, the embryo was gold covered and scanned.

Descriptive terminology of the eggshell follows Clark Sellick (1997), but using shell, *sensu* Moscona (1950), instead of capsule. The elucidation and naming of eggshell (= exochorion) and internal layers (endochorion, vitelline membrane and exuvia) is based on Snodgrass (1935), Moscona (1950), Bedford (1970), Furneaux and Mackay (1976) and Gullan and Cranston (2010). Since the scanned embryo was fully formed and enclosed by a membrane identified as the cast embryonic exuvia, we follow Bedford (1970) and refer to it as a pharate first instar nymph.

Examined material.— *Paraphasma paulense* Rehn, 1918

All first instar nymphs were obtained through eggs laid by females collected in the BBS. The studied material was deposited at MZSP. The list follows below, with eggs and nymphs listed after their progenitor females.

BRAZIL, São Paulo state, Salesópolis municipality, Estação Biológica de Boraceia, 23°39'14"S, 45°53'21"W, 900m: one adult female (pinned with both wings opened) (MZSP 176), 20–21.iv.2012, four eggs (two for SEM), two egg shells (after nymph eclosion) and two first instar nymphs, F.F. Albertoni col.; one adult male (MZSP 178), 19–23.vi.2012, F.F. Albertoni col.; one adult female (pinned with left wings opened) (MZSP 175), 19–23.x.2012, two first instar nymph, two eggs and one egg shell (after nymph eclosion), F.F. Albertoni and M.A. Ulysséa col.; one adult male (pinned with left wings opened) (MZSP 177), 14–18.i.2013, F.F. Albertoni col.; one adult female (parasitized by two *Forcipomiya paulista* Falaschi, Albertoni and Fusari, 2014) (MZSP 375), 15–20.ii.2013, one egg, F.F. Albertoni col.; one adult female (MZSP 374) and three adult males (MZSP 174, 179, 180), 08–12.iii.2013, one egg, F.F. Albertoni col.

Morphology

Paraphasma paulense Rehn, 1918: 191–192 + pl. X figs. 3–5. Holotype male, Brazil, São Paulo state, Cantareira, ii.1914, E. Garbe. Deposited at the Academy of Natural Sciences of Philadelphia, type no. 5347 (photograph examined in Phasmida Species File Online, Brock et al. 2017).

Table 1. Measurements of the egg of *Paraphasma paulense* Rehn, 1918. External measurements, n=5; opercular and internal measurements, n=3.

Measurement (mm)		Measurement (mm)	
Length	3.2–3.4	Micropylar plate length	1.3–1.6
Width	1.4–1.6	Micropylar plate width	0.4–0.5
Height	1.5–1.7	Internal micropylar plate length	1.1–1.2
Operculum height	1.2	Internal micropylar plate width	0.3–0.4
Operculum width	0.9	Internal median line length	0.3

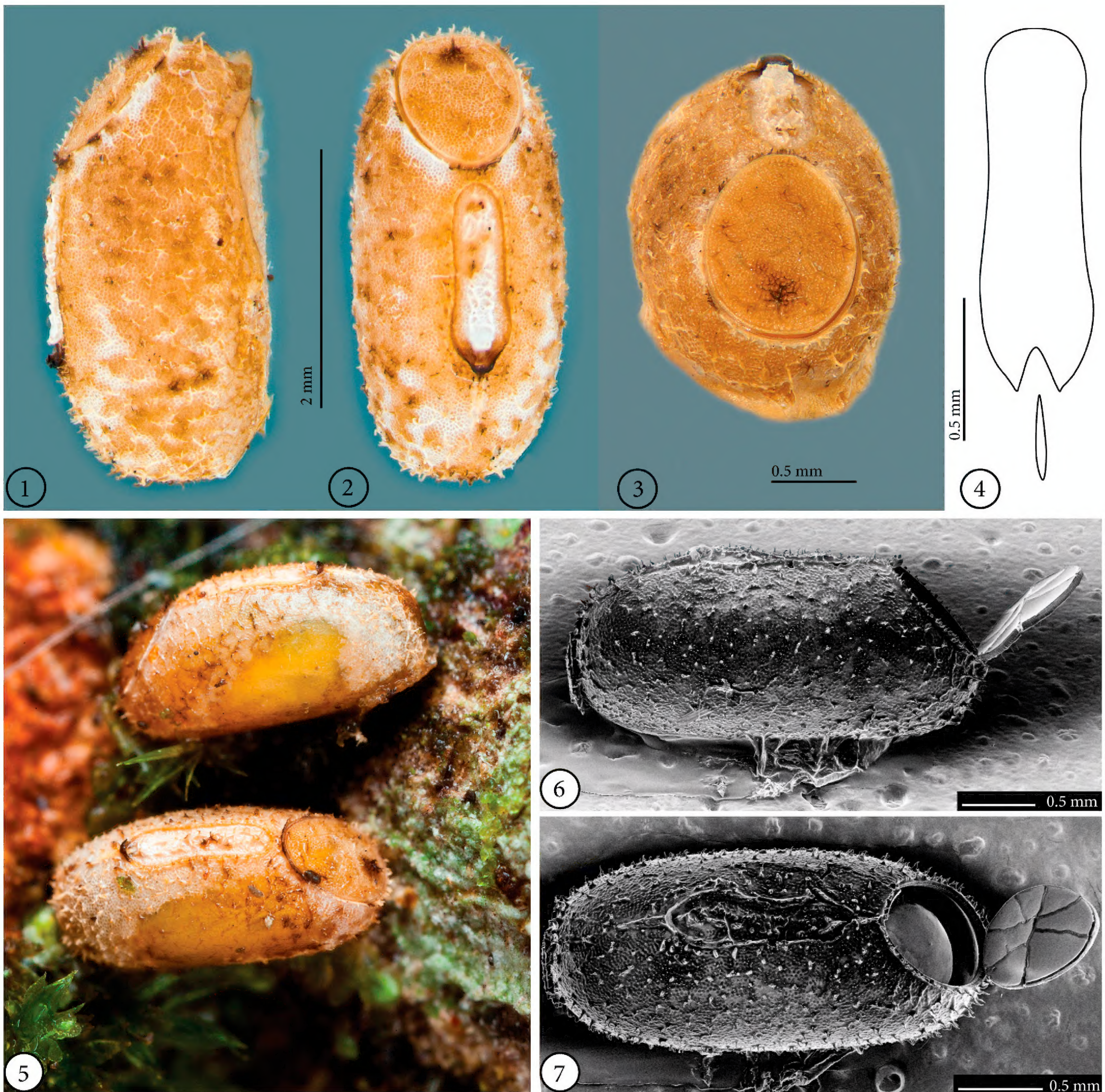
Egg (Figs 1–15; Table 1).— *Description*: Elongate, sub-cylindrical, almost round in cross-section, dorsal and lateral surfaces gently convex, ventral surface (where the egg is glued to substrate) and polar area slightly flat. Operculum oval and indistinctly convex, strongly inclined dorsally (opercular angle of approximately 45°), almost touching anterior margin of micropylar plate. Surfaces of shell and operculum covered with minute sinuous projections, often hooked (Figs 6–10). Micropylar plate elongate, approximately 3 $\times$ longer than wide, extending for more than 1/3 of egg length, slightly widened in posterior region, bearing along its entire length elongate and sinuous ridges; ridges posteriorly culminating on a considerably large hump, occasionally bearing small projections similar to those covering shell. Micropylar cup positioned on posterior margin of micropylar plate, continuous to a short median line hardly visualized externally, but very conspicuous on the inner surface of shell. Internal micropylar plate open (Fig. 4).

Internal layers (Figs 10–15) and *pharate first instar nymph inside egg* (Figs 16–18): Pharate first instar nymph enclosed by three layers within shell (exochorion), identified as endochorion (outermost), vitelline membrane, and cast off embryonic exuvia (innermost). Endochorion smooth (Figs 10–11), attached to exochorion only in the micropylar stalk. Surface of vitelline membrane covered with minute spherical structures (Figs 14–15). Head integument of pharate first instar nymph bearing sparse small setae (Figs 12–14).

First instar nymph (Figs 24–25).— *Description*. *Color*: When alive, body yellow to pale brown, head laterally with longitudinal dark stripe, eyes similar to head color or slightly darker, with a longitudinal darker stripe medially. General coloration pale cream after fixed.

Head: Surface smooth but with few small and strongly sparse setae; length approximately 1.5 $\times$ the width across eyes. Eyes prominent, ellipsoid, 0.4 $\times$ as long as head. Ocelli absent. Antennae 9-segmented, as long as body, homogeneously covered with fine setae, when alive whitened around last 1/4 apicad, distal flagellomere as long as previous four together and darker at apex; scape 1.5 $\times$ longer than pedicel, cylindrical. Frons bearing several very small setae; clypeus, labrum and distal maxillary and labial palpomere with many tiny setae.

Thorax: Dorsally convex; ventrally flat or slightly concave, slightly rigged laterally. Surface smooth, but with several small and sparse setae. Pronotum narrower and 0.7 $\times$ longer than head, with one longitudinal furrow medially and one transversally nearer anterior margin; openings of the pair of pronotal glands present, anteriorly. Mesonotum more than 2 $\times$ longer than pronotum or metanotum. Metanotum slightly longer than median segment. Sternal region of thorax indistinct. Wing buds absent. Legs bearing relatively sparse, short and fine setae, but denser than on thorax and abdomen. Femora flat ventrally. Anterior femur slightly curved proximally, as in adult specimens. First tarsomere as long



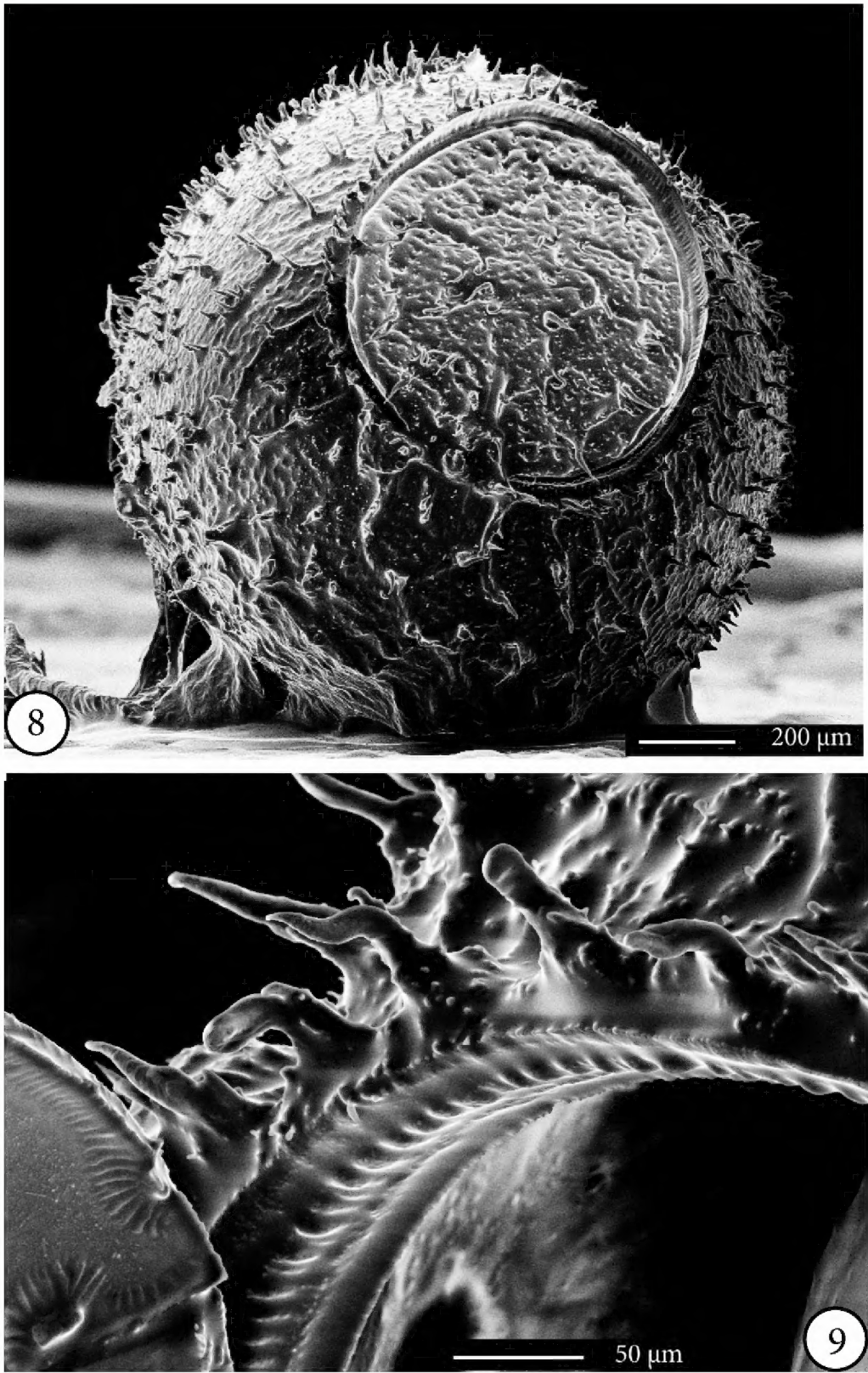
Figs 1–7. *Paraphasma paulense* Rehn, 1918 egg. 1–3. Lateral, dorsal and frontal (opercular) view, respectively; 4. Schematic illustration of internal micropylar plate; 5. Eggs detached from plastic container with pharate nymph inside; 6, 7. SEM of egg with open operculum; 6. Lateral view; 7. Dorsolateral view showing endochorion and internal face of operculum.

as or longer than remainder of tarsus. Hind leg the longest, almost as long as body. Arolium well developed, gibbous, almost as long as tarsal claws.

Abdomen: Ten visible tergites. Dorsally with four longitudinal rows of sparse short setae, laterally with several setae. Median segment as long as tergite II. Tergite IX the shortest. One pair of small cerci at apex, as long as tergite X, covered by very small setae.

Adult female (Figs 19–23, 26–27; Table 2).— **Diagnosis:** Relatively slender stick insects; body length ranging around 55–60 mm. Live

specimens predominantly olivaceous, densely spotted in pale yellow or light brown, with a pale yellow dorsomedian line extending along head, pro- and mesothorax; body ventrally light-colored, with a dark median line extending along the body except subgenital plate; tegmina and costal region of hind wing dark olive or brownish green with venation vivid light green. Antennae long, nearly as long as body. Head usually bearing a pair of dorsomedian, large black spines, gently curved forward, absent in some specimens. Tegmina reaching intermediate region of median segment, bearing a pronounced, sharp spine; hind wings well-developed.



Figs 8–9. *Paraphasma paulense* Rehn, 1918 egg. SEM images: 8. Frontal view with operculum; 9. Detail of opercular socket and shell surface projections.

Table 2. Measurements of *Paraphasma paulense* Rehn, 1918 female (n=4).

Length (mm)		Length (mm)	
Body	55.6–59	Hind wing	37.9–43.1
Head	3.1–3.6	Profemur	12–13.5
Antennae	51–57.5	Mesofemur	7.2–8.6
Pronotum	3.6–3.8	Metafemur	11.1–13
Mesonotum	5.6–6.8	Protibia	10.9–12.3
Metanotum	7.5–8	Mesotibia	6–7
Median segment	3–3.3	Metatibia	9.7–10.8
Tegmina	10.6–11.5	Cercus	1.5–1.8

Subgenital plate distinctly short, not reaching posterior margin of tergite IX.

Description. *Color:* General pattern dark olive or brown. Head, pro- and mesothorax with dorsal pattern of dark shades of brown, olive or grey, densely spotted in lighter color such as pale yellow or bright brown; dorsomedian longitudinal line in the same lighter color. Metanotum and abdominal terga shiny dark brown, except

for three or four final tergites, usually in paler greenish brown. Ventral coloration variable, usually in shades of shiny buff; some individuals greenish on head, pro- and mesosternum; a dark brown ventromedian line extending longitudinally along the entire body except subgenital plate. Tegmina dark grey or olive with venation in vivid light green. Costal region of hind wing in the same general pattern, but with some sparse, light pale green regions; anal region translucent with venation brown. Femora and tibiae variegated, with the same colors as pro- and mesonotum; tarsi dark grey or brown. When alive, with the same general coloration, but more vivid, especially in the green and olivaceous areas.

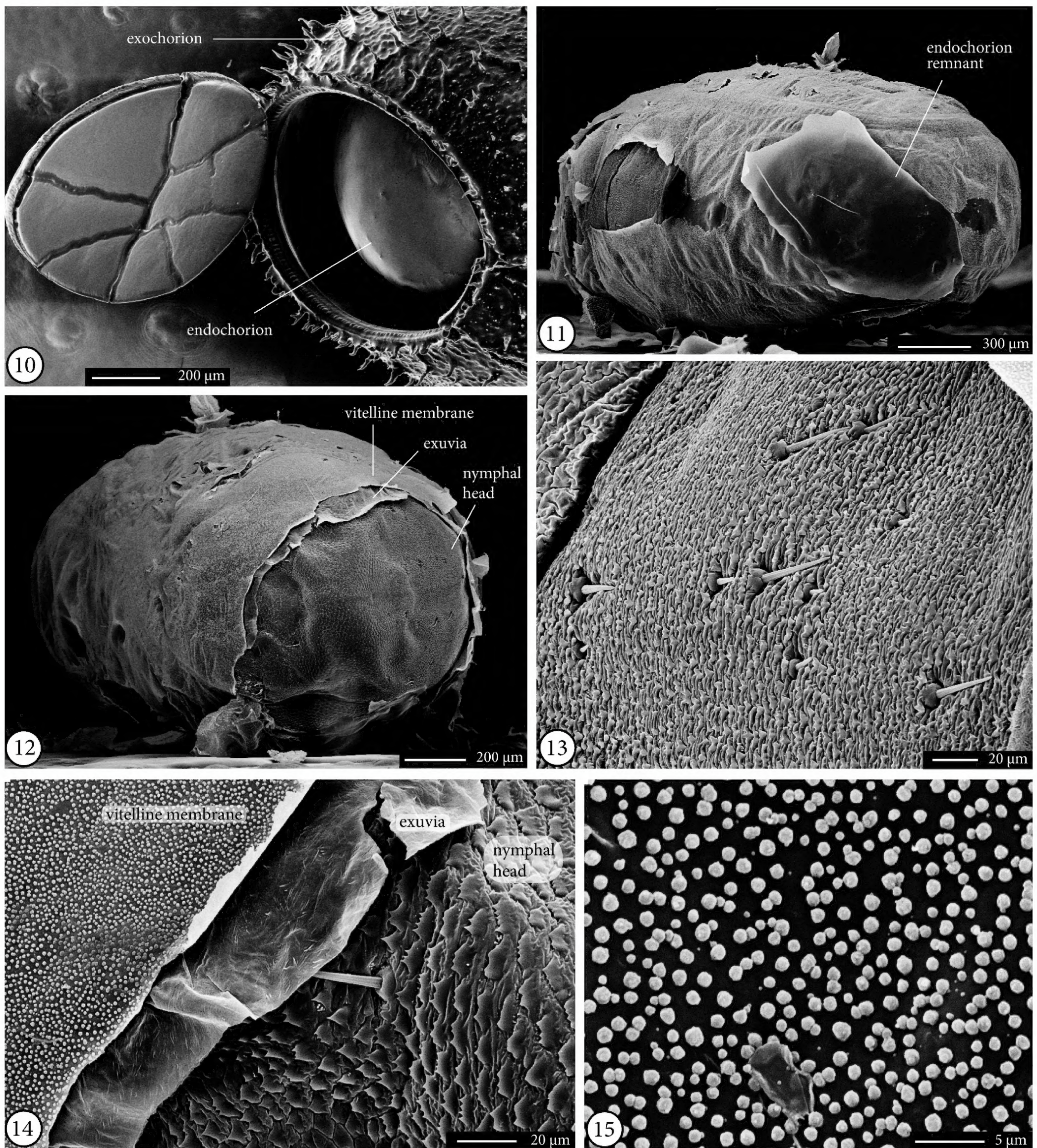
Head: About 1.1× longer than wide, dorsoventrally compressed, sub-rectangular in dorsal view; surface smooth except for one pair of dorsomedian large black spines, gently curved forward, completely absent in some individuals. Eyes strongly prominent, 0.4× as long as head, pale orange to bright russet, with diffuse dark stains. Ocelli small, usually in the same color as eyes. Antennae long, almost reaching tip of abdomen, covered with fine setae; scape dorsoventrally compressed; pedicel and flagellomeres cylindrical; pedicel 0.7× as long as scape; basal and apical regions of flagellum with shortest flagellomeres, median region with longest ones.

Thorax: Dorsally convex; ventrally flat or slightly concave. Surface generally smooth, with few sparse granules close to the lateral margins of mesonotum. Pronotum sub-rectangular, approximately as long as, and slightly narrower than head, 1.5× longer than wide, with two discreet transversal furrows, one near the anterior margin and one medially; openings of the pair of defensive glands very conspicuous on anterolateral corners, crescent-shaped. Mesonotum as wide as, and 1.6–1.8× longer than pronotum, narrowed towards posterior margin; a pair of slightly elevated ridges originating in anterior margin and extending posteriorly and convergently until base of tegmina. Metanotum and median segment combined 1.6–1.9× longer than mesonotum, as wide as posterior end of mesonotum. Metanotum approximately 2.5× longer than median segment; metanotum and median segment smooth. Metaepisternum with a longitudinal ridge accompanying its ventral margin.

Wings: Tegmina elongate, almost 3× longer than wide, reaching intermediate region of median segment; anterior third bearing a pronounced, approximately conical spine, basally broad and apically acute. Hind wing well-developed and elongate, reaching or surpassing posterior margin of abdominal tergite VIII.

Legs: Profemur curved and compressed basally; anterodorsal and posteroventral carinae markedly raised, especially the former. Profemur and protibia bearing weakly raised ventromedian carina. Femur and tibia of mid- and hind legs approximately trapezoidal in cross section, with distinct carinae on the four edges, ventromedian carina absent, ventral surface slightly convex. All tibiae with conspicuous area apicalis. Each femur slightly longer than the respective tibia. Profemur 1.5–1.8× longer than mesofemur and slightly longer than metafemur. Pro- and metabasitarsus slightly longer than the following three tarsomeres combined, mesobasitarsus similar in length to the following three tarsomeres combined.

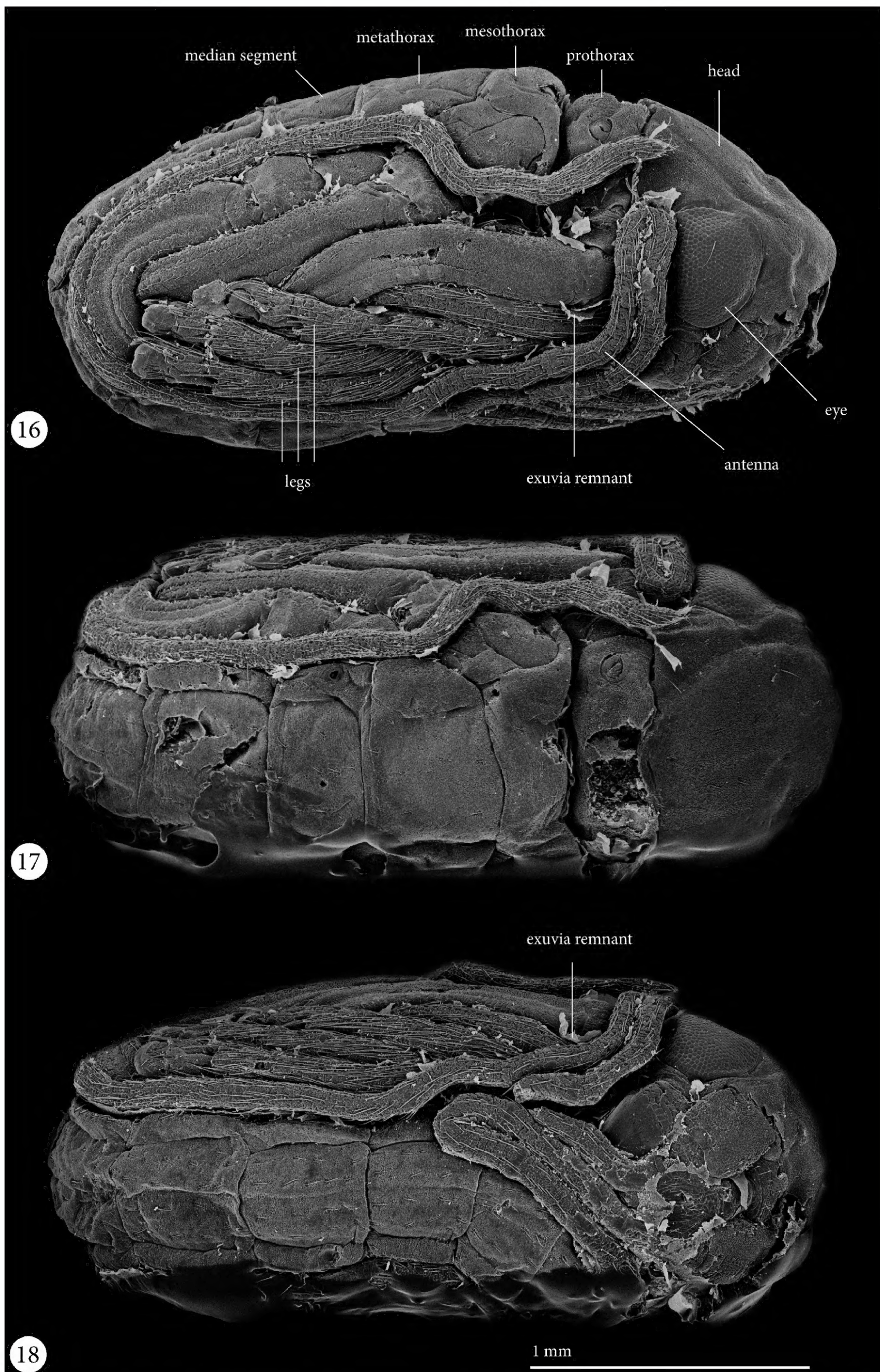
Abdomen: Dorsal and ventral surfaces smooth. Tergites II–V parallel-sided, of equal length and width. Tergites VI and VII narrowing towards posterior end; tergite VI of similar length in relation to preceding ones; tergite VII 0.8× as long as VI; tergite VIII slightly expanding towards posterior end; 0.6× as long as VII; tergites IX and X of similar length and 0.6× as long as VIII; tergite IX strongly narrowing towards posterior end; tergite X parallel-sided, posterior margin rounded in dorsal view and horseshoe-shaped in



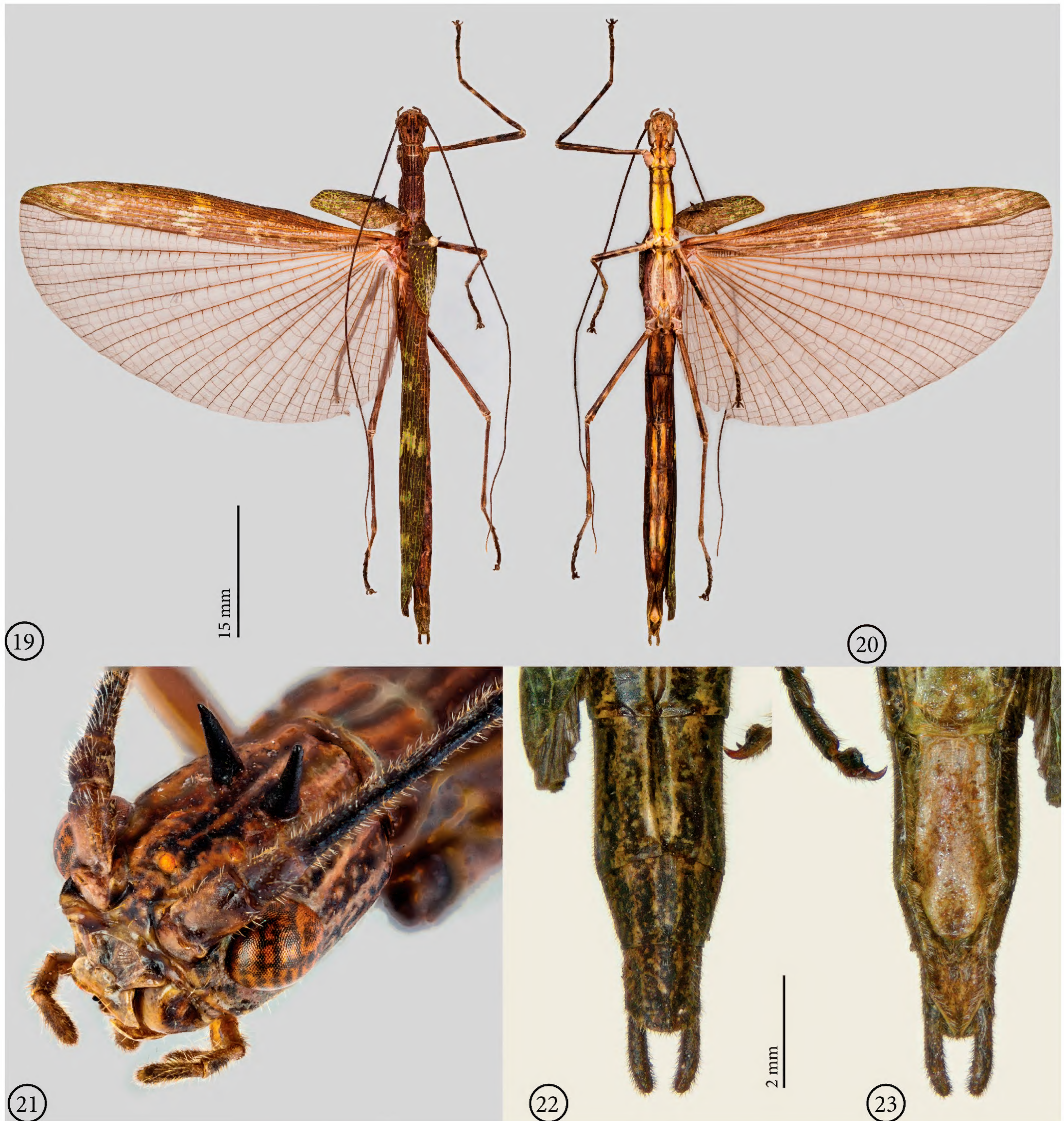
Figs 10–15. *Paraphasma paulense* Rehn, 1918 ultrastructure of egg and internal content. 10. Operculum opened and endochorion surface; (11, 12, 13, 14) pharate first instar nymph: 11. Enclosed in vitelline membrane; 12. With part of the head exposed evidencing nymph integument, embryonic exuvia and vitelline membrane; 13. Integument; 14. Detail of Figure 12; 15. Detail of vitelline membrane surface.

caudal view. Supra-anal plate not visible. Tergites VII–X inconspicuously carinated, degree of carination varying among individuals; tergites VII and VIII longitudinally bicarinated, IX and X single-

carinated. Sternites II–VI with a dark, thin, longitudinal carina. Preopercular organ inconspicuous, as a discreet, round, bilobed protuberance on posterior region of sternite VII. Subgenital plate



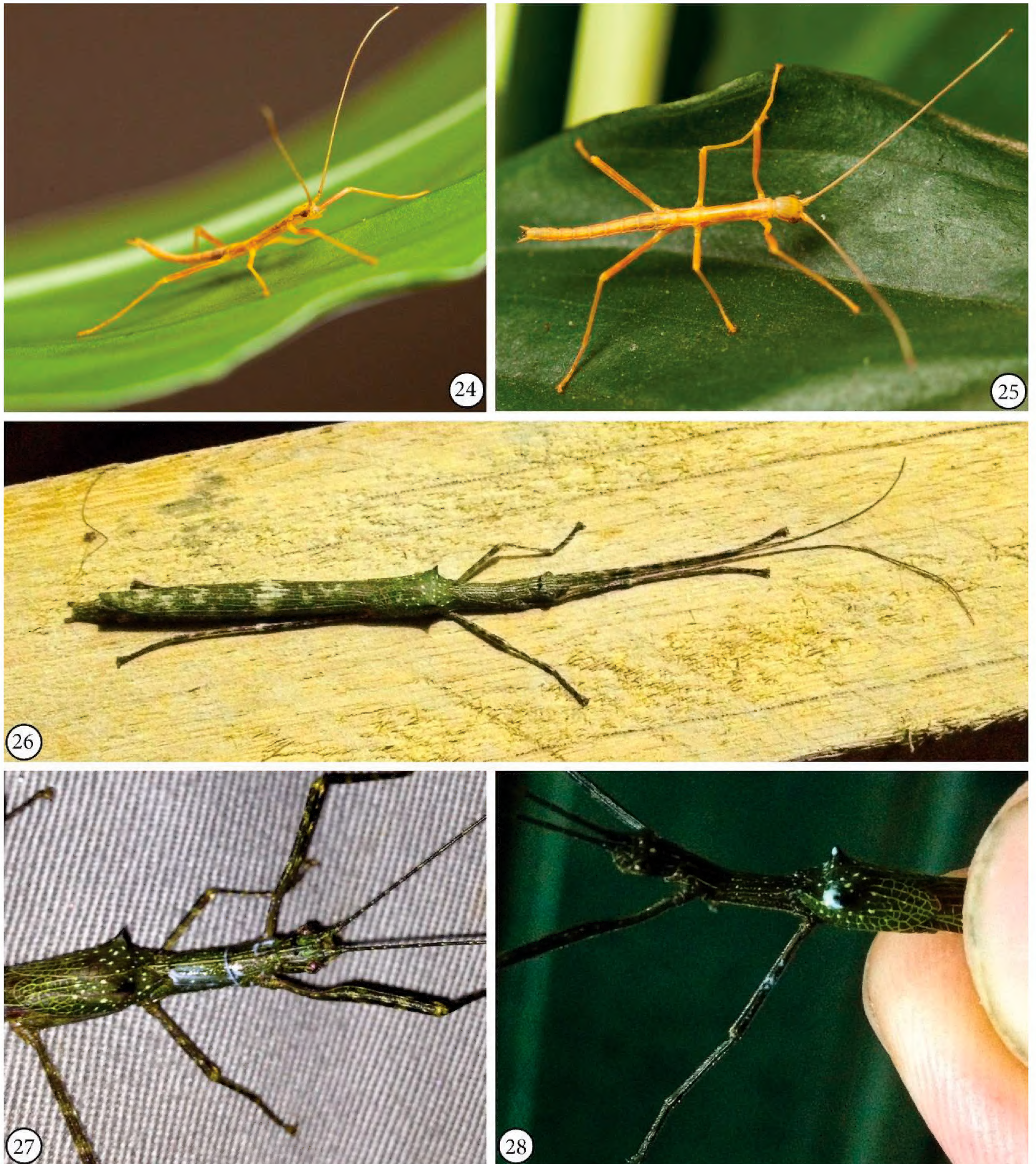
Figs 16–18. *Paraphasma paulense* Rehn, 1918 habitus of first instar nymph inside the egg. 16. Lateral view; 17. Dorsal view; 18. Ventral view.



Figs 19–23. *Paraphasma paulense* Rehn, 1918 adult female. 19, 20. Habitus, dorsal and ventral views; 21. Head; 22, 23. End of abdomen, dorsal and ventral views.

short, not reaching posterior margin of tergite IX; widening in anterior two-thirds, posterior region rounded and concave, apex weakly pointed. Gonapophyses prominent, projecting over posterior margin of subgenital plate and surpassing end of tergite IX. Cerci elongate and relatively stout, width constant from base to dull apex. Ventral surface of segments VIII–X, as well as structures originating from them, covered with minute setae.

Biology.— No food plant could be determined, though many species were offered to adults and nymphs. Although plant species were not previously identified, among them were: *Alchornea* sp. (Euphorbiaceae), *Eugenia uniflora*, *Psidium cattleianum* (Myrtaceae), and two more unidentified species of Myrtaceae, *Olyra* sp. (Poaceae), *Serjania* sp. (Sapindaceae) and *Schinus terebinthifolius* (Anacardiaceae). The only plant species that one female fed on was *Humiriastrum* sp.



Figs 24–28. *Paraphasma paulense* Rehn, 1918 live specimens. 24, 25. First instar nymph, lateral and dorsal views; 26. Adult female resting; 27. Adult female after releasing the defensive substance, with secretion spread over the thorax; 28. Adult male after spraying the defensive substance backwards, with secretion accumulated on the tegmina spines and mesofemur.

(Humiriaceae), which caused it intestinal dysfunction after 12 hours – faeces were strongly soft and long, not typically broken into solid droplets.

Cryptic eggs were laid and strongly glued on the sticks of the plant branches given for feeding purpose. They took from 104 to 126 days to hatch ($n=5$). First instar nymphs did not feed on any plant offered and were fixed.

Male and female adults secreted a white substance through the prothoracic glands as a result of contact stimulation, as when held or strongly disturbed (Figs 27–28). Secretion was not observed in first instar nymphs. Adults could spray the substance backwards, in the direction of disturbance (Fig. 28), or let it ooze over the surface of the prothorax (Fig. 27).

Dossey et al. (2012) tested a spiroketal substance from the stick insect *Asceles glaber* Günther, 1938 (Diapheromeridae: Necrosciinae) as a repellent of the ant *Solenopsis invicta* Buren, 1972 and the mosquito *Aedes aegypti* Linnaeus, 1762. Tested spiroketal of the *A. glaberi* defensive spray demonstrated some efficiency only as a contact repellent. However, the quinolone from the defensive spray of *Oreophoetes peruana* (Saussure, 1868) (Diapheromeridae: Diapheromerinae) did repel *Monomorium pharaonis* (Linnaeus, 1758) ants, *Schizocosa ceratiola* (Gertsch & Wallace, 1935) spiders, *Periplaneta americana* (Linnaeus, 1758) cockroaches, and irritated the skin of frogs (*Pseudacris crucifer* (Wied-Neuwied, 1838)) (Eisner et al. 1997). Other stick insect species are mentioned as producing defensive substances that can irritate sensitive mucus membrane of the eyes, nose and mouth (Brutlag et al. 2011, Dossey et al. 2012).

Despite these records one female of *P. paulense* was collected with two females of the midge *Forcipomyia paulista* Falaschi, Albertoni & Fusari, 2014 attached to it, one on the prothorax and one on the base of right tegmen (Falaschi et al. 2014). The secreted substance of *P. paulense* was not chemically evaluated, but immediately after being released by the insect, it smelled fresh-sour and had a sharp odour somewhat reminding of camphor. When deeply sniffed (by FFA), the secretion caused a few sneezes or transitory nose irritation, although the smell was not unpleasant. This “experiment” was performed four times with the same result.

Morphological remarks

Apart from a study dealing specifically with color polymorphism in *Anisomorpha buprestoides* (Stoll, 1813) (Conle et al. 2009), and some very brief morphological observations on the first instar nymph of *Tithonophasma tithonus* (Lima et al. 2013), to the authors’ knowledge the description of a newly hatched nymph in the family Pseudophasmatidae is herein presented for the first time. Even for Phasmatodea as a whole, morphological descriptions of first instar nymphs have been unusual or strongly brief. Nevertheless, more studies and comparisons of first instar nymphs of different stick insect species should allow a better understanding of structures of taxonomic relevance for the order.

With this background, we could only make general comparisons with first instar nymphs of species belonging to families other than Pseudophasmatidae. Remarkable differences, which may be of taxonomic importance for high-level taxonomic categories, were noted on: antennae length; body pubescence; shape of femora and tibiae; proportions between the sizes of body parts; coloration of body and appendages.

First instar nymph of *Orthomeria kangi* Vallotto et al., 2016 (Aschiphasmataidae: Aschiphasmatinae) (Vallotto et al. 2016) differs from the one of *P. paulense* on the color of the whole body

and body parts and (*P. paulense* parenthetical): body setal vestiture dense, black and relatively long on all body and body appendages (scarce, yellowish and relatively short); pronotum apparently bulged (not bulged); mesonotum less than 2× longer than pronotum or metanotum (more than 2× longer); median segment longer than metanotum (slightly shorter); anterior femur straight (slightly curved proximally); posterior tibia arched (straight).

First instar nymph of *Phryganistria bachmaensis* (Ta and Hoang, 2004) (Phasmatidae: Clitumninae) differs from the one of *P. paulense* by the color of the whole body and body parts and (*P. paulense* parenthetical): antenna strongly shortened (long, almost body length), 8-segmented (9-segmented). First instar nymph of *Phryganistria tamdaoensis* Bresseel & Constant, 2014 was only briefly characterized (Bresseel and Constant 2014). It differs from *P. paulense* in general body color mostly green but with central portion of profemur and proximal part of meso- and metafemora reddish brown, and in the very short antenna.

Probably the most remarkable difference between the first instar nymph and the adult of *P. paulense* is the tergite of the median segment presenting a distinctly lower degree of fusion to the metanotum in the nymph (although a suture is still easily discernible separating both regions in the adult). Pictures available in Vallotto et al. (2016) indicate that this difference is also observed between first instar nymph and adult of *O. kangi*, suggesting that a progressive increase in the fusion between metanotum and median segment tergite throughout post-embryonic development may be a widespread condition in Phasmatodea. In *P. paulense*, the first instar nymph also differs from the adult in general coloration of body, proportions between size of body segments, and (adult parenthetical): ocelli absent (present); antennae 9-segmented, scape cylindrical, distal flagellomere as long as previous four together (antennae with more than 40 segments, scape dorsoventrally compressed, distal flagellomeres much shorter than the median ones); abdomen dorsally with four longitudinal rows of sparse, short setae (absent).

Paraphasma paulense presents weak sexual dimorphism, with both sexes exhibiting well-developed hind wings, and females being only slightly stouter and longer than males. Additionally, the pair of cephalic dorsomedian large spines (Fig. 21), present in most females, was absent in all males examined, as well as in the holotype (image available in Brock et al. 2017).

Based on the present study of the female of *P. paulense*, as well as on the examination of males also collected at the BBS (listed in the “Examined material” section), it is noticeable that this species presents some clear morphological differences in relation to the diagnostic characters described for *Paraphasma* (see Introduction). While the meso- and metafemora of *Paraphasma* typically lack strong carination, in *P. paulense* they are approximately trapezoidal in cross section, with the edges corresponding to distinct carinae. Additionally, the tegmina of *P. paulense* are considerably long, reaching the middle of median segment – in contrast to the characteristic scale-shaped tegmina of *Paraphasma*, which do not surpass the posterior margin of metanotum (see photographs of the lectotype and paralectotypes of the type species of the genus, *Paraphasma marginale* Redtenbacher, 1906, available in Brock et al. 2017).

Only a few brief descriptions and simple illustrations are available for eggs of *Paraphasma*, in Clark Sellick (1997) and Zompro (2004). Based on these studies and on the examination of eggs of *P. marginale* by the first author, it is clear that *P. paulense* strongly differs in egg morphology from other representatives of the genus. The most remarkable differences regard opercular angle (approximately 45° towards dorsal surface in *P. paulense*; not inclined in

other *Paraphasma* representatives), size and shape of micropylar plate (larger and much more elongate in *P. paulense*) and shell ornamentation (small sinuous projections in *P. paulense*; strongly bristled in other *Paraphasma* representatives). Remarkably, *P. paulense* also differs in the egg-laying strategy, gluing the eggs to the substrate, in contrast to the more common strategy among Phasmatodea (including *P. marginale* – PICM, pers. obs.) of simply dropping the eggs to the ground.

With the aforementioned differences between *P. paulense* and other representatives of the genus, both in adult and egg morphology, we present evidence in favour of its future removal from *Paraphasma*. The transference of *P. paulense* to another genus will be the subject of a taxonomic study in preparation by Oskar Conle (pers. comm.).

Acknowledgements

We are sincerely thankful to Fernando Campos De Domenico for aid in the species identification, to Oskar V. Conle for a discussion on the taxonomy of *P. paulense*, and to Marcelo Duarte (MZSP) for access to MZSP facilities. We are also grateful to the Fundação de Amparo à Pesquisa do Estado de São Paulo for financial support to PI Chiquetto-Machado (grant no. 2015/08808-2) and FF Albertoni (grant no. 2012/02441-1).

References

- Bates HW (1865) Descriptions of fifty-two new species of Phasmidae from the collection of Mr. W. Wilson Saunders, with remarks on the family. Transactions of the Linnean Society of London 25: 321–359. <https://doi.org/10.1111/j.1096-3642.1865.tb00187.x>
- Bedford GO (1970) The development of the egg of *Didymuria cuilescens* (Phasmatodea : Phasmatidae : Podacanthinae) – embryology and determination of the stage at which first diapause occurs. Australian Journal of Zoology 18:155–169. <https://doi.org/10.1071/ZO9700155>
- Bresseel J, Constant J (2014) Giant sticks from Vietnam and China, with three new taxa including the second longest insect known to date (Phasmatodea, Phasmatidae, Clitumninae, Pharnaciini). European Journal of Taxonomy 104: 1–38. <https://doi.org/10.5852/ejt.2014.104>
- Brock PD, Büscher T, Baker E (2017) Phasmida Species File Online. Version 5.0/5.0. <http://phasmida.speciesfile.org> [accessed 22 Mar 2017]
- Brunner von Wattenwyl K (1907) IX. Tribus Clitumnini, X. Tribus Lonchodini, XI. Tribus Bacunculini. In: Brunner von Wattenwyl K, Redtenbacher J (Eds) Die Insektenfamilie der Phasmiden. Wilhelm Engelmann, Leipzig, 181–338. [Tafel VII–XV]
- Brutlag AG, Hovda LR, Della Ripa MA (2011) Corneal ulceration in a dog following exposure to the defensive spray of a walking stick insect (*Anisomorpha* spp.). Journal of Veterinary Emergency and Critical Care 21: 382–386. <https://doi.org/10.1111/j.1476-4431.2011.00654.x>
- Burmeister H (1838) Handbuch der Entomologie, Zweiter Band. Theod. Chr. Friedr. Enslin, Berlin, 397–756.
- Clark Sellick JT (1997) Descriptive terminology of the phasmid egg capsule, with an extended key to the phasmid genera based on egg structure. Systematic Entomology 22: 97–122. <https://doi.org/10.1046/j.1365-3113.1997.d01-30.x>
- Clark Sellick JT (1998) The micropylar plate of the eggs of Phasmida, with a survey of the range of plate form within the order. Systematic Entomology 23: 203–228. <https://doi.org/10.1046/j.1365-3113.1998.00056.x>
- Conle OV, Hennemann FH, Dossey AT (2009) Survey of the color forms of the southern two-striped walkingstick (Phasmatodea: Areolatae: Pseudophasmatidae: Pseudophasmatinae: Anisomorpha), with notes on its range, habitats, and behaviors. Annals of the Entomological Society of America 102: 210–232. <https://doi.org/10.1603/008.102.0204>
- Dossey AT, Whitaker JM, Dancel MCA, Meer RKV, Bernier UR, Gottardo M, Roush WR (2012) Defensive spiroketals from *Asceles glaber* (Phasmatodea): absolute configuration and effects on ants and mosquitoes. Journal of Chemical Ecology 38: 1105–1115. <https://doi.org/10.1007/s10886-012-0183-x>
- Eisner T, Morgan RC, Attygalle AB, Smedley SR, Herath KB, Meinwald J (1997) Defensive production of quinoline by a phasmid insect (*Oreophoetes peruana*). Journal of Experimental Biology 200: 2493–2500.
- Falaschi RL, Albertoni FF, Fusari LM (2014) A new species of *Forcipomyia* (*Microhelea*) Meigen (Insecta: Diptera: Ceratopogonidae) from the Neotropical region. Zootaxa 3878: 379–389. <https://doi.org/10.11646/zootaxa.3878.4.4>
- Fundação Florestal (2017) Governo do Estado de São Paulo, Secretaria do Meio Ambiente, Parque Estadual da Serra do Mar. <http://www.parqueestadualserradomar.sp.gov.br/pesm/> [accessed 31 March 2017]
- Furneaux PJS, Mackay AL (1976) The composition, structure and formation of the chorion and the vitelline membrane of the insect egg-shell. In: Hepburn HR (Ed.) The insect integument. Elsevier, Amsterdam, 157–176.
- Goldberg J, Bresseel J, Constant J, Kneubühler B, Leubner F, Michalik P, Bradler S (2015) Extreme convergence in egg-laying strategy across insect orders. Scientific Reports 5: 7825. <https://doi.org/10.1038/srep07825>
- Gray GR (1835) Synopsis of the species of insects belonging to the family of Phasmidae. Longman, Rees, Orme, Brown, Green, and Longman, London, 48.
- Gullan PJ, Cranston PS (2010) The insects: an outline of entomology. 4th Edition. Blackwell Publishing, Oxford, 505 pp.
- Heleodoro RA, Mendes DMM, Rafael JA (2017) Studies on Brazilian Pseudophasmatidae (Phasmatodea) with the description of a new species of *Agrostia* Redtenbacher and new records for *Metriophasma* Uvarov and *Parastratocles* Redtenbacher. Revista Brasileira de Entomologia 61: 170–177. <https://doi.org/10.1016/j.rbe.2017.03.003>
- Hennemann FH, Conle OV, Perez-Gelabert DE (2016) Studies on Neotropical Phasmatodea XVI: Revision of *Haplopodini* Günther, 1953 (rev. stat.), with notes on the subfamily Cladomorphinae Bradley and Galil, 1977 and the descriptions of a new tribe, four new genera and nine new species (Phasmatodea: “Anareolatae”: Phasmatidae: Cladomorphinae). Zootaxa 4128: 1–211. <https://doi.org/10.15468/v0awld>
- Kirby WF (1904) Notes on Phasmidae in the collection of the British Museum (Natural History), South Kensington, with descriptions of new genera and species. No. II. Annals and Magazine of Natural History 7: 429–449. <https://doi.org/10.1080/00222930408562475>
- Kumagai AE, Fonseca NG (2009) Uma nova espécie de Cladomorphus Gray, 1835 (Phasmatidae, Cladomorphinae) de Minas Gerais, Brasil. Revista Brasileira de Entomologia 53: 41–44. <http://dx.doi.org/10.1590/S0085-56262009000100011>
- Lima AR, Kumagai AE, Neto FCC (2013) Morphological and biological observations on the stick insect *Tithonophasma tithonus* (Gray, 1835) (Phasmida: Pseudophasmatidae: Pseudophasmatinae). Zootaxa 3700: 588–592. <http://dx.doi.org/10.11646/zootaxa.3700.4.7>
- Moscona A (1950) Studies of the egg of *Bacillus libanicus* (Orthoptera, Phasmidae). I. The egg envelopes. Quarterly Journal of Microscopical Science 91: 183–193.
- Redtenbacher J (1906) I. Tribus Bacillini, II. Tribus Obrimini, III. Tribus Pygrrhynchini, IV. Tribus Ascepasmini, V. Tribus Anisomorpha, VI. Tribus Phasmini, VII. Tribus Heteropterygini, VIII. Tribus Phyllini. In: Brunner von Wattenwyl K, Redtenbacher J (Eds) Die Insektenfamilie der Phasmiden. Wilhelm Engelmann, Leipzig, 1–180 [Tafel I–VI]
- Redtenbacher J (1908) XII. Tribus Phibalosomini, XIII. Tribus Acrophyllini, XIV. Tribus Necrosiini. In: Brunner von Wattenwyl K, Redtenbacher J (Eds) Die Insektenfamilie der Phasmiden. Wilhelm Engelmann, Leipzig, 339–572 [Tafel XVI–XXVII]
- Rehn JAG (1918) On Dermaptera and Orthoptera from South-Eastern Brazil. Transactions of the American Entomological Society 44: 181–222.
- Sellick J (1997) The range of egg capsule morphology within the Phasmatodea and its relevance to the taxonomy of the order. Italian Journal of Zoology 64: 97–104. <https://doi.org/10.1080/11250009709356178>

- Snodgrass RE (1935) Principles of insect morphology. McGraw-Hill, New York, 667 pp.
- Toledo Piza S (1936) Os Phasmidas do Museu Paulista. I. Phasmidae, Bacillinae. Revista de Entomologia 6: 280–292.
- Toledo Piza S (1937) Os Phasmidas do Museu Paulista. II. Phasmidae, Phasminae. Revista de Entomologia 7: 1–8.
- Toledo Piza S (1938) Novos Phasmidas do Brasil e da Argentina (Orth.). Revista de Entomologia 9: 1–11.
- Toledo Piza S (1944) Cinco novas espécies de Phásmidas do Brasil. Anais da Escola Superior de Agricultura “Luiz de Queiroz” 1: 43–58. <https://doi.org/10.1590/S0071-12761944000100004>
- Vallotto D, Bresseel J, Heitzmann T, Gottardo M (2016) A black-and-red stick insect from the Philippines – observations on the external anatomy and natural history of a new species of *Orthomeria*. Zookeys 559: 35–57. <https://doi.org/10.3897/zookeys.559.6281>
- Westwood JO (1859) Catalogue of Orthopterous insects in the collection of the British Museum. Part I. Phasmidae. British Museum, London, 195 pp.
- Zompro O (2001) A generic revision of the insect order Phasmatodea: The New World genera of the stick insect subfamily Diapheromeridae: Diapheromerinae = Heteronemiidae: Heteronemiinae sensu Bradley and Galil, 1977. Revue Suisse de Zoologie 108: 189–255. <https://doi.org/10.5962/bhl.part.79626>
- Zompro O (2004) Revision of the genera of the Areolatae, including the status of *Timema* and *Agathemera* (Insecta, Phasmatodea). Abhandlungen des Naturwissenschaftlichen Vereins in Hamburg (NF) 37: 1–327.